Hybridization between serrate leaf *Juniperus monosperma* and smooth leaf *J. scopulorum* in the Guadalupe Mountains, NM, USA: evidence from DNA sequencing and leaf essential oils

Robert P. Adams and Sam T. Johnson
Baylor University, Biology Department, Utah Lab, Baylor University, Waco, TX 76798, USA
robert_Adams@baylor.edu

Richard D. Worthington
UTEP herbarium, University of Texas at El Paso, El Paso, TX 79968

and

George M. Ferguson
Herbarium, University of Arizona, 1140 E. South Campus Dr., Tucson, AZ 85721-0036

ABSTRACT

Two unusual *J. scopulorum* trees were discovered in the Guadalupe Mtns., NM and analyses of petN-psbM (cpDNA) confirmed that had chloroplasts (cp) of *J. monosperma*. nrDNA (ITS)sequencing revealed 25 SNPs between *J. monosperma* and *J. scopulorum.* 18 SNPS were analyzed and all SNPs were heterozygous in the 2 unusual plants, implying they are hybrids. In addition, DNA from a 2010 herbarium voucher at UTEP was successfully extracted and sequenced. It contained *J. monosperma* cp and all 18 ITS SNPs were heterozygous, showing it was also a hybrid. Addition analyses of the leaf volatile oils of *J. monosperma, J. scopulorum* plus the 2 putative hybrid trees, confirmed they are hybrids between *J. monosperma* and *J. scopulorum* in the Guadalupe Mtns., NM. Published on-line www.phytologia.org *Published on-line www.phytologia.org Phytologia 102(3):131-142 (Sept 21, 2020). ISSN 030319430.*
KEY WORDS: *Juniperus scopulorum, J. monosperma, nrDNA, petN-psbM,* hybridization, leaf essential oils, Guadalupe Mountains, New Mexico.

Juniperus, in North America, has been the subject of numerous studies on hybridization using morphological data (Fassett, 1944, 1945a, 1945b, Hall 1952; Van Haverbeke 1968; Schurtz 1968).Later studies involved the use of chemical data (Flake et al. 1978; Adams 1983,Palma-Otal et al. 1983; Adams and Kistler 1991, Adams 2013a,b). Recently, DNA sequence data has been used in the study of hybridization between *J. occidentalis* and *J. osteosperma* (Terry et al. 2000; Terry 2010); *J. maritima* and *J. scopulorum* (Adams 2015a, b); *J. scopulorum* and *J. blancoi* (Adams et al. 2020); *J. arizonica* and *J. coahuilensis* (Adams 2017).

The recent study on hybridization and introgression between *J. scopulorum*, in the United States and *J. blancoi* in Mexico utilized nrDNA (ITS region) and cp DNA (petN-psbM, trnS-trnG) sequences for several populations of both species (Adams et al. 2020). Analysis of *J. scopulorum* in the Guadalupe Mountains, NM, found typical *J. scopulorum* DNA, except for two *J. scopulorum* trees with nrDNA intron sites that supported introgression from *J. blancoi* in Mexico (Adams et al. 2020). Additional sampling discovered two other *J. scopulorum* trees that seemed unusual and upon DNA analyses, they appear to be of hybrid origin with nearby *J. monosperma* trees. Because *J. scopulorum* is in the entire leaf margined clade and *J. monosperma* is a member of the serrate leaf junipers clade (Adams 2014), the taxa are, phylogenetically, somewhat remote. Thus, hybridization between these species would see less likely than between, for example, two serrate junipers (*J. arizonica*, *J. coahuilensis*, Adams 2017) or two entire leaf junipers (*J. blancoi*, *J. scopulorum*, Adams et al. 2020).

The purpose of this report is to present both DNA data (nrDNA, cpDNA sequences) and analysis of the leaf essential oils of *J. monosperma and J. scopulorum* to investigate the possible hybrids between *J. monosperma* and *J. scopulorum* in the Guadalupe Mtns., NM, USA.

MATERIAL AND METHODS

Plant material

***J. scopulorum*, Guadalupe Mtns., NM**

Lab Acc. *Robert P. Adams 15602*, ex *Richard Worthington 28617,* UTEP Herbarium accession 58749, Devil's Den Spring, Guadalupe Mtns. 32° 02' 3.12" N, 104° 16' 0.12" W. 2103m(6000ft), 5 Sept. 1999, Eddy County, NM

Lab Acc. *Robert P. Adams 15603*, ex *Richard Worthington 28673*, UTEP Herbarium accession 58750 North Fork, Big Canyon, Guadalupe Mtns. 32° 02' 3.12" N, 104° 45' 0.12" W. 1828m (6000ft), 6 Sept. 1999, Eddy County, NM**.**

Lab acc. *Adams 15783,* *ex George M. Ferguson4624,* with J. Ferguson, Riparian woodland. limestone. male tree, 2 trunks each 30 cm dbh, 9 m tall; bark longitudinally plated, pollen cones forming. Associated species: *Pinus ponderosa var. brachyptera, Pinus edulis, Juniperus deppeana, Quercus muehlenbergii, Quercus grisea, Acer grandidentatum, Berberis haematocarpa, Arbutus xalapensis var. texana, Dasylirion leiophyllum, Agave parryi*. Lincoln National Forest, Guadalupe Mountains, Dark Canyon, S of Klondike Gap near confluence Hooper Canyon, 0.2 mi (by FR 307) E jct County Road 412 (FR 69), just inside USFS boundary. TRS: T25S R21E sec 26 SE1/4, 32° 6' 0" N, 104° 46' 15.6" W.1920m (6300 ft.),3 November 2019, Eddy County, NM

Lab Acc. *Robert P. Adams 15799*, ex *George M. Ferguson 4649*

male tree, 41 cm dbh, 8.5 m tall; bark rough longitudinally plated, dark red beneath, pollen cones just beginning to form, Lincoln National Forest, Guadalupe Mtns., upper Devil's Den canyon, on limestone, Pinyon-oak-juniper woodland w *J. deppeana* and *Pinus* sp., 32° 02' 18.96' N, 104° 48' 5.04" W, 2170m (7120 ft), 19 Jan 2020.Eddy County, NM

***J. monosperma*, Guadalupe Mtns., NM**

Lab Acc. Robert P. Adams 15781ex George M. Ferguson4616 with J. Ferguson, Riparian woodland. limestone. female tree, multiple trunks ca. 20 cm dbh, 4 m tall, bark longitudinally furrowed, cones dark blue with light bloom, 1-seeded. Associated species: *Pinus ponderosa, Pinus edulis, Juniperus deppeana, Quercus muehlenbergii, Quercus grisea, Dasylirion leiophyllum, Agave parryi,* Lincoln National Forest, Guadalupe Mountains, Dark Canyon, 0.4 mi N confluence Goat Canyon on Cougar Road 412 (FR 69). TRS: T25S R22E sec 17 SE1/4, 32° 7' 57"N, 104° 43' 9.84" W. 1768m (5820 ft.), 20 Oct 2019, **Eddy County, NM.**

Lab Acc. *Robert P. Adams 15805, 15806*exColl. *George M. Ferguson 4660, 4661*

female tree, multiple trunks < 10 cm dbh each, 2.5 m tall, bark longitudinally furrowed, cones dark blue with light bloom, 1-seeded (rarely 2), Lincoln National Forest, Guadalupe Mtns., 0.6 mi (by NM 137) W jct FR 540 Guadalupe Ridge Road, at milepost 14.5, on limestone, Pinyon-oak-juniper woodland w *J. deppeana* and *Pinus* sp.32° 9' 43.56" N, 104° 47' 6.36" W, 1868m (6130 ft), 19 Jan 2020.Eddy County, NM

Lab Acc. *Robert P. Adams 15807,* *George M. Ferguson 4662*

male tree, multiple trunks, 15 cm dbh each, 3.5 m tall, bark longitudinally furrowed, pollen cones formed not shedding pollen yet, Lincoln National Forest, Guadalupe Mtns., 1.5 mi (by NM 137) E jct FR 540 Guadalupe Ridge Road, at milepost 16.5, on limestone, Pinyon-oak-juniper woodland w *J. deppeana* and *Pinus* sp. 32° 11' 9.6" N, 104° 46' 6.6" W, 1797m (5895 ft), 19 Jan 2020. County Eddy, NM,

***J. scopulorum* x *J. monosperma*, Guadalupe Mtns., NM**

Lab Acc. *Robert P. Adams 15601*, Coll. Richard Worthington 36160, UTEP Herbarium accession 80150, scale lvs with few very small teeth. otherwise foliage as *scopulorum*. Devil's Den Canyon,

Guadalupe Mtns. 32° 02' 15" N, 104° 47' 54.24" W. ca 2164m (7100ft), 18 July 2010, Eddy County, NM

Lab Acc. Robert P. Adams 15787, ex George M. Ferguson4628 with J. Ferguson
scale lvs with few very small teeth. otherwise foliage as scopulorum. Riparian woodland. limestone. male tree, ca. 10 cm dbh, 3 m tall; bark longitudinally plated. Associated species: *Pinus ponderosa var. brachyptera, Pinus edulis, Juniperus deppeana, Quercus muehlenbergii, Quercus grisea, Acer grandidentatum, Berberis haematocarpa, Arbutus xalapensis var. texana Dasylirion leiophyllum, Agave parryi,* Lincoln National Forest, Guadalupe Mountains, Dark Canyon, S of Klondike Gap near confluence Hooper Canyon, 0.2 mi (by FR 307) E jct County Road 412 (FR 69), just inside USFS boundary. TRS: T25S R21E sec 26 SE1/4, 32° 6' 0" N, 104° 46' 15.6" W. 1920m. (6300 ft.), 3 November 2019, **Eddy County, NM**

Lab Acc. *Robert P. Adams 15804*, *ex* Coll. *George M. Ferguson 4658,*
scale lvs with few very small teeth. otherwise foliage as scopulorum. male tree, 90 cm dbh, 12 m tall; bark rough longitudinally plated, reddish brown beneath, few old pollen cones falling, no new cones yet, Lincoln National Forest, Guadalupe Mtns., upper Devil's Den canyon, on limestone, Pinyon-oak-juniper woodland w *J. deppeana* and *Pinus* sp. T26S, R21E, Sec 16 SE ¼ , 32° 2' 17.16" N, 104° 48' 2.52" W, 2182m (7155 ft), 19 Jan 2020, Eddy County, NM

Voucher specimens are deposited at the Herbarium, Baylor University (BAYLU).

Isolation of Oils- Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20ºC until analyzed. The extracted leaves were oven dried (100ºC, 48 h) for determination of oil yields.

Volatile oil Analyses- Oils from 10-15 trees of each of the taxa were analyzed and average values are reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details, out of print, free pdf: www.juniperus.org). Identifications were made by library searches of our volatile oil library (Adams, 2007, www.juniperus.org), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

DNA analysis - One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN-psbM), (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used), 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS (ITS+42F, ITS-57R) and petN-psbM (petN5F, psbM111R) primers utilized. Due to the presence of an indel (1 bp deletion at site 194 in *J. scopulorum*), sequences for the mon x scop hybrids were not readable from site 194 forward. A reverse primer was designed (ITS765r, ATC GCA CTT CAT TCT TTT Tm 49.7˚C) and then synthesized by IDT (Integrated DNA Technologies, Inc.), San Diego, CA. This primer was used to obtain clean sequences in hybrids for sites S2-S16. The reverse primer, ITS-57R, was to read site S25.

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.).

RESULTS AND DISCUSSION

Sequencing nrDNA (ITS) resulted in 1270 bp and comparison between *J. monosperma* and *J. scopulorum* revealed 25 SNPs plus 5 indels. Due the difficulty of sequencing sites 18 - 24, these sites were not analyzed. These large number of differences between *J. monosperma* and *J. scopulorum* underscore the magnitude of phylogenetic differences between the serrate leaf junipers and the smooth (entire) leaf margined junipers. In fact, these clades are thought to have migrated to North America (NA) at different times, and by different routes. The serrate leaf junipers appear to have migrated to NA ca. 47 to 30.5 Mya from Europe via the NALB (North America Land Bridge) from Europe to Iceland, Greenland, Nova Scotia, thence into the dry Madrean -Tethyan vegetation zones in southwestern US and Mexico (see Fig. 1.4, Adams 2014). The smooth leaf margined junipers are very closely related to *J. sabina*/ *J. davurica* in the China - eastern Russian area of the eastern hemisphere, thence across the BLB (Bering Land Bridge) ca. 17.6 to 5.5 Mya (see Fig. 1.7, Adams 2014).

The ITS sequences clearly revealed that 3 unusual plants, appearing to be a variant of *J. scopulorum* in the field, were heterozygous for the 18 ITS variable sites (Table 1). These 3 plants (15601, 15787 and 15804, appearing morphologically as *J. scopulorum* in the field, are hybrids by their ITS DNA.

Table 1. SNPs from nrDNA(ITS) and cp DNA classification of *J. scopulorum, J. monosperma* and *J. monosperma* x *J. scopulorum* from Guadalupe Mtns., NM.na = not available.

Acc. # &	pollen pat.	nuc. mat.	nrDNA (ITS) 18 of 25 informative sites[1]																
field id species	cp	ITS	S1 179	S2 205	S3 257	S4 285	S5 315	S6 338	S7 348	S8 350	S9 352	S10 353	S11 368	S12 406	S13 422	S14 432	S15 545	S16 614	S17 615	S25 1173
15602 scop	scop	scop	C	C	C	T	A	C	T	T	G	T	C	G	T	A	T	C	T	T
15603scop	scop	scop	C	C	C	T	A	C	T	T	G	T	C	G	T	A	T	C	T	T
15783 scop	scop	scop	C	C	C	T	A	C	T	T	G	T	C	G	T	A	T	C	T	T
15799 scop	scop	scop	C	C	C	T	A	C	T	T	G	T	C	G	T	A	T	C	T	T
15601 scop	mon	MxS	C/T	na	na	C/T	A/T	C/T	G/T	C/T	A/G	C/T	C/T	A/G	C/T	A/G	G/T	C/T	G/T	na
15787scop	mon	MxS	C/T	C/T	C/T	C/T	A/T	C/T	G/T	C/T	A/G	C/T	C/T	A/G	C/T	A/G	G/T	C/T	G/T	C/T
15804scop	mon	MxS	C/T	C/T	C/T	C/T	A/T	C/T	G/T	C/T	A/G	C/T	C/T	A/G	C/T	A/G	G/T	C/T	G/T	C/T
15781 mon	mon	mon	T	T	T	C	T	T	G	C	A	C	T	A	C	G	G	T	G	C
15807 mon	mon	mon	T	T	T	C	T	T	G	C	A	C	T	A	C	G	G	T	G	C
15782 mon	mon	mon	T	T	T	C	T	T	G	C	A	C	T	A	C	G	G	T	G	C
15805 mon	mon	mon	T	T	T	C	T	T	G	C	A	C	T	A	C	G	G	T	G	C
15806 mon	mon	mon	T	T	T	C	T	T	G	C	A	C	T	A	C	G	G	T	G	C

[1]S1,179:xGCGGACA,S2,205:xGCTGGAGGG; S3,257:xGAATGCCG; S4,285: xCCCGCGG; S5,315: xTCTGGATC;S6, 338: xCGAAACGA; S7,348: CGAAACGAx; S8,350(y): CGAAACGAxTy; s9,352(z), S10,353(!): CGAAACGAxTyTz!;S11,368:xCCCTGCTC; S12,406: xTCCCCCGT; S13,422:xCATGGCTC; S14, 432: xTCGTGTGC; S15,545: xTGTTCAGG;S16,614: CTCTCCCTx; S17,615(y): CTCTCCCTxy; S25, 1173: xGCGGGCA;

Sequencing petN-psbM yielded 5 informative SNPs that resolved the *J. monosperma* cp from *J. scopulorum* cp. Thus, all samples could be readily scores as having *J. monosperma* or *J.* scopulorum cp (Table 1). The three tress that were field identified, morphologically, as '*J. scopulorum*', were all hybrids in their nrDNA, and each had the *J. monosperma* cp (Table 1).

Examination of the leaf margins (40x) revealed they were smooth, except for very small teeth near the bottom of the leaf margins. Plant 15601 specimen did not have seed cones and plants 15787 and 15804 were males, so no seed cones were available to observe.

If these 3 plants are indeed F_1 hybrids, it seems odd that the morphology is so similar to*J. scopulorum*. So, we decided to investigate the leaf essential oils as they have proven useful to detect hybridization in *Juniperus* (Flake et al. 1978; Adams 1983, Palma-Otal et al. 1983; Adams and Kistler 1991, Adams 2013a, b).

The volatiles oils of *J. monosperma* and *J. scopulorum* have been published (RPA) from several locations from our (RPA) lab: *J. monosperma* (Adams 1994; Adams et al. 2014a, 2014b) and *J. scopulorum* (Adams 2009, 2015a). However, it is important to analyze oils from trees in the vicinity of the putative hybrids. The volatile leaf essential oil (EO) of *J. monosperma* is dominated by α-pinene (62.3 - 75.8%), with moderate amounts of β-phellandrene (5.3-7.1%), elemol (0.7 - 3.1%), β-eudesmol (0.9 - 8.4%) and 8-α-acetoxyelemol (0.8 - 1.0%). The EO of *J. scopulorum* is dominated by sabinene (40.7 - 48.4), with moderate amounts of α-pinene (2.6 - 2.7%), limonene (2.1 - 1.8%), β-phellandrene (1.7), terpinen-4-ol (1.7 - 3.7%),pregeijerene B (6.3 - 8.7%), germacrene D-4-ol (1.6 - 1.7%) and 8-α-acetoxyelemol (4.5 - 4.9%).

The EO of *J. monosperma* and *J. scopulorum* differ distinctly (Table 2) in 15 compounds (bold face): α-thujene, α-pinene, sabinene, β-pinene, α-terpinene, limonene, β-phellandrene, camphor, coahuilensol, terpinen-4-ol, pregeijerene B, germacrene B, germacrene D-4-ol, α-cadinol and 8-α-acetoxyelemol. Often, the concentrations of EO components is intermediate between parents of hybrids (Adams and Tsumura 2012; Adams and Stoehr 2013), and this is the case for 10 compounds: α-thujene, α-pinene, sabinene, β-pinene, γ-terpinene, cis-sabinene hydrate, trans-sabinene hydrate, terpinen-4-ol, germacrene D-4-ol, and 8-α-acetoxyelemol (Table 2). These data provide strong support that the unusual plants (15797, 15804) are hybrids. It might be noted that the two *J. scopulorum* EO (15783, 15799, Table 2) represent the two chemotypes present in *J. scopulorum* (and *J. virginiana*). This appears to be due to a single gene that appears to turn on the production of aromatic ethers synthesized in the phenylpropanoid pathway that is separate from the terpenoid pathway (von Rudloff 1975; Adams et al. 1981). Note the presence of safrole, methyl eugenol, (Z)-isoeugenol, and elemicin (scop 15804, Table 2), which were co-extracted in the terpenoids. It appears a high aromatic ethers type plant (cf. 15804) is not a parent of the hybrids (15787 and 15804) because they are both devoid of aromatic ethers (i.e., safrole, methyl eugenol, (Z)-isoeugenol, and elemicin).

Ten of the EO components are transgressive (i.e., concentration of a compound it larger (or smaller) than the concentration in either parent). Nine transgressive compounds have a higher concentration in the hybrids than in either parent:α-fenchene, δ-2-carene, δ-3-carene, limonene, β-phellandrene, terpinolene, cis-p-menth-2-en-1-ol, trans-p-menth-2-en-1-ol, α-terpineol and abietadiene (Table 2). Only one, pregeijerene B has a lower concentration than either parent (Table 2). Analyses of the inheritance of terpenoids in this study versus in *Cryptomeria japonica* and *Pseudotsuga menziesii*, reveals that intermediate inheritance (10 cpds., this study) is comparable (Table 3) to *C. japonica* (7 cpds.) and *P. menziesii* (11, 2 plus 8 dominant or recessive cpds.). The number of transgressive cpds. (higher conc.) in this study (10) is similar to *C. japonica* (5 cpds.) and *P. menziesii* (9, 4) and the number of transgressive cpds. (lower conc.) in this study (1) is low compared to *C. japonica* (5 cpds.) and *P. menziesii* (5,5).

Another facet of mixing germplasms in hybrids genomes is that some biochemical pathways can produce novel (new) compounds because the enzymes from both parents may be present in a synthesis region in the cell. For example, an acetylation enzyme from one parent may act upon α-terpineol to produce α-terpinyl acetate as seen in Table 2 (both parents have α-terpineol but not α-terpinyl acetate, as it is only found in the hybrids). The hybrids had 18 "new" compounds NOT found in either parent (Table 2, 3)! This compares to one (1) in *C. japonica* and none (0) in *P. menziesii*. The hybrids analyzed from *C. japonica* and *P. menziesii* (Adams and Tsumura2012; Adams and Stoehr 2013) were all derived from infra-specific crosses in which the genomes were very similar. Thus, no new compounds resulted from those crosses.

Table 3. Inheritance of terpenoids in hybrids in this study compared with inheritance in literature reports.

Mode of inheritance in hybrids vs. number of compounds	This study	*Cryptomeria japonica* (Adams and Tsumura (2012)	*Pseudotsuga menziesii* (Adams and Stoehr 2013)	
			wide cross	narrow cross
Concentration intermediate between *monosperma* and *scopulorum* plants sampled.	10	7	11	2 (+ 8 dominant/ recessive)
Transgressive, higher conc. than found in *monosperma* or *scopulorum* plants sampled.	10	5	9+ % oil yield	4 + % oil yield
Transgressive, lower conc. than found in either *monosperma* or *scopulorum* plants sampled.	1	5	5	5
Novel cpds. not found in either *monosperma* or *scopulorum* plants sampled.	18	1	0	0

The distribution of *Juniperus monosperma* in the Guadalupe Mts. is from the north, where it occurs at mid-elevations of the adjacent Sacramento Mts., NM, southward along The Rim in the northern portion of the Guadalupe Mts., predominately at 5800 – 6200 ft. It grows in a low-profile pinyon-juniper woodland with the associated *Juniperus deppeana*, *Pinus edulis* and *Quercus grisea*. Outlying plants extend onto the base of the western escarpment with scattered individuals to ca. 5000 ft., in semidesert grassland and a few plants as low as 4400 ft. in Chihuahuan desert-scrub. Apparently, *J. monosperma* is rare in the canyons or pediment of the southern escarpment of the Guadalupe Mts. (in Texas) although populations extend farther south to the adjacent Diablo and Apache Mts. Whereas *J. monosperma* is tolerant of xeric environments, the more mesic habitat requirements of *Juniperus scopulorum* limit it to riparian canyon bottoms and north-facing slopes of canyons. The southernmost population for the species is in the Guadalupe Mts., where the distribution of *J. scopulorum* is disjunct from the upper portions of the Sacramento Mts. to the north. In the Guadalupe Mts., *J. scopulorum* occurs at upper elevations, primarily at 6300-7200 ft., in riparian woodlands in the north-central portion of the Guadalupe Mts., while some individuals extend down into the largest canyons of the southern escarpment (in McKittrick canyon, Texas) to ca. 6000 ft. with the associated *Juniperus deppeana*, *Pinus*, *Quercus*, *Acer* and *Arbutus*.

It appears that *J. scopulorum* and *J. monosperma* are separated elevationally, and by habitat such that locally, the species are essentially allopatric within the Guadalupe Mts. In other regions across their ranges, which are widely overlapping, these two species can be locally sympatric (e.g. Gila National Forest, NM, and Mogollon Rim, AZ). From our observations, the nearest *J. monosperma* to the hybrid *J. scopulorum* tree in Dark Canyon at 6300 ft., is 3.5 air miles N near the jct. of NM 137 and FR 520 at 6100 ft., or 3.5 mi ENE in lower in Dark Canyon at 5800 ft., or 4.0 air miles WNW on NM 137 at 5800 ft. The *J. monosperma* nearest to the two hybrid *J. scopulorum* trees in Devil's Den Canyon at 7150 ft., is 4.5 air miles NNE below The Rim near El Paso Gap at 5450 ft., or 5.0 air miles NNE on NM 137 at 5800 ft. The sparse juniper-oak-pine woodland in upper Dog Canyon (ca. 2 air miles W of the hybrid trees, at the mouth of Devil's Den Canyon) is apparently *J. deppeana* without *J. monosperma* at 6100-6200 ft. However, this general discussion does not eliminate the possibility that a few scattered *J. monosperma* trees may occur nearer the hybrids.

Inasmuch as *J. deppeana* is widespread in the Guadalupe Mts., and occurs at a wide range of elevations (5500 – 8000 ft.) and, thus, is sympatric with *J. scopulorum* and *J. monosperma*, these latter occur in different, specific habitats. Along the southern pediment and lower eastern slopes of the

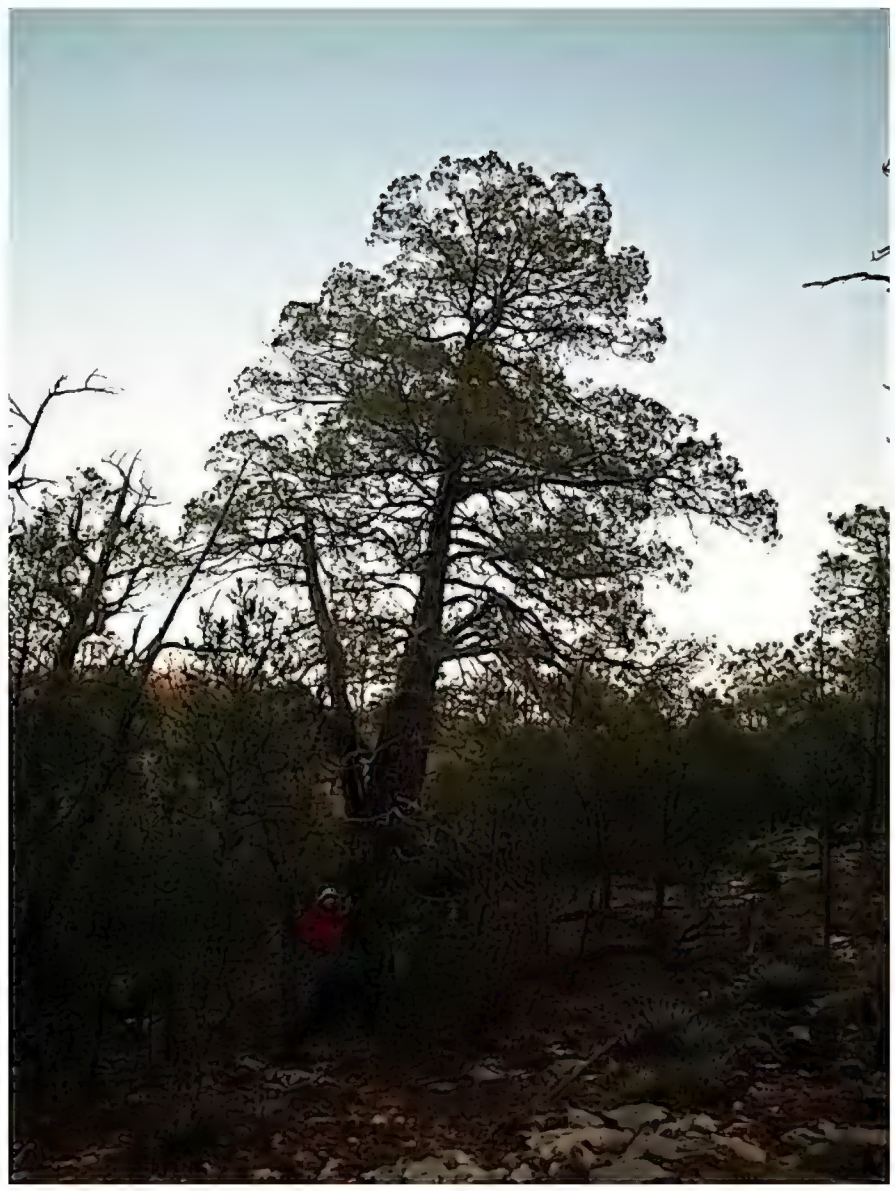

Fig. 1(left). Putative mono x scop hybrid tree with round crown shape (RPA 15804, GF4658). Note the the several large side branches and irregularly spaced, odd angled and balls of foliage on branches.

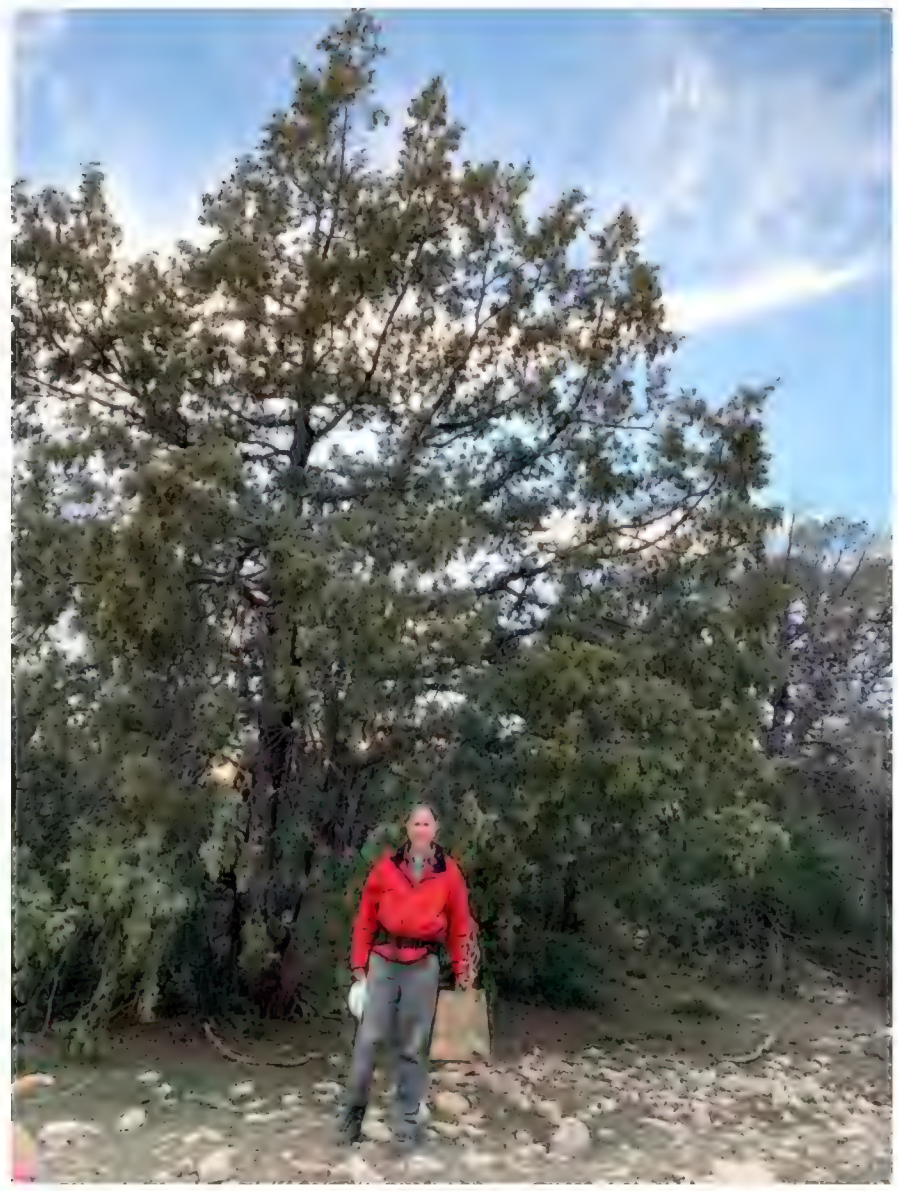

Fig. 1(right) Typical crown of *J. scopulorum*. Note the strong central axis, pyramidal shape, and the regularly spaced, uplifted side branches.

Figure 2 (left). Leaves and bark of mono x scop hybrid tree, Lab 15804 (GF4658). Note dark green leaves and short, strong branchlets.

Fig. 2 (right). Leaves and bark of scopulorum, GF 4649. Note glaucous leaves and longer branchlets.

Guadalupe Escarpment, *Juniperus pinchotii* is also present, though generally not sympatric with either *J. scopulorum* or *J. monosperma*. Additionally, *J. pinchotii* sheds its pollen in the fall prior to November (Ferguson personal observation; Adams 2014), in contrast the other junipers in the area, pollen is shed is in the spring (Adams 2014). Comparison of the putative hybrid, (RPA 15804, GF4658) (Fig. 1,left) with typical J. scopulorum (Fig. 1, right) reveals that the hybrid has a round crown (as do the branch tips) vs. the pyramidal crown, and elongated branch tips. In addition, the hybrid has several large side branches compared to fairly uniform and equally spaced, uplifting side branches in *J. scopulorum*. The foliage of the hybrid is more compact, and greener that that of *J. scopulorum* (Fig. 2, left vs. right). The bark of the hybrid is twisted and exfoliation in very thick strips vs. thinner strips in *J. scopulorum* (Fig. 2, left vs. Taken together, EO components being intermediate, transgressive and newly found, DNA complementary in the hybrid, and morphology, these provide strong evidence that the putative hybrids are indeed hybrids between *J. monosperma* and *J. scopulorum.* Additional research is needed to more fully understand this evolutionary event.

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Table 2. Compositions of the leaf oils of *J. monosperma* (mono), *and J. scopulorum* (scop) and putative hybrids. Green highlight = intermediate concentration between *monosperma* and *scopulorum*; Yellow = transgressively larger concentration than either *monosperma* or *scopulorum*; Tan = transgressively smaller in hybrids than in putative parents; Blue = cpd. not found in either putative parent species, Compounds that appear to separate the parents are in boldface. Aromatic ethers (only found in 15799) are in purple.

KI	compound	mono 15781	mono 15807	mono x scop 15787	mono x scop 15804	scop 15783	scop 15799
921	tricyclene	t	t	t	t	t	t
924	**α-thujene**	**t**	**t**	**0.9**	**0.6**	**1.6**	**1.1**
932	**α-pinene**	**62.3**	**75.8**	**21.5**	**13.2**	**2.7**	**2.6**
945	**α-fenchene**	**t**	**t**	**0.5**	**0.6**	**t**	**t**
946	camphene	0.3	t	t	t	t	t
953	thuja-2,4(10) diene	t	t	-	-	-	-
969	**sabinene**	**0.1**	**0.2**	**23.1**	**18.6**	**48.4**	**40.7**
974	**β-pinene**	**1.2**	**1.8**	**0.8**	**0.9**	**t**	**t**
988	myrcene	0.7	1.6	3.0	3.4	1.5	0.8
1001	δ-2-carene	t	t	5.1	0.2	0.2	t
1002	α -phellandrene	0.4	0.5	t	0.3	t	t
1008	δ-3-carene	0.3	t	7.9	15.0	t	t
1014	**α -terpinene**	**t**	**t**	**0.8**	**0.6**	**1.4**	**0.8**
1020	p-cymene	0.3	0.3	0.5	0.9	0.3	0.1
1024	**limonene**	**-**	**-**	**4.6**	**1.8**	**2.1**	**1.8**
1025	**β-phellandrene**	**5.1**	**7.1**	**4.5**	**13.4**	**1.7**	**1.7**
1132	limonene oxide	-	-	-	**0.3**	-	-
1044	(E)-β-ocimene	t	t	0.2	0.2	t	t
1054	γ-terpinene	0.5	0.5	1.4	1.0	2.3	1.4
1065	cis-sabinene hydrate	t	t	0.6	0.6	1.3	0.7
1086	terpinolene	0.7	0.9	1.5	1.9	1.3	0.8
1097	trans-sabinene hydrate			0.5	0.3	1.2	0.6
1097	linalool	0.3	t	0.4	0.3	-	-
1100	n-nonanal	t	t	-	-	-	-
1101	cis-thujone (= α-thujone)	-	-	-	-	-	t
1108	1,3,8-p-menthatriene	-	-	-	-	t	-
1112	trans-thujone (= β-thujone)	-	-	-	-	t	t
1118	cis-p-menth-2-en-1-ol	t	t	**0.4**	**0.5**	0.3	0.2
1122	α-campholenal	t	t	t	t	-	-
1136	**trans-p-menth-2-en-1-ol**	t	t	**0.3**	**0.3**	0.1	t
1138	gejgerene	-	-	-	-	t	t
1140	trans-verbenol	-	-	**t**	-	-	-
1141	**camphor**	**0.3**	**0.2**	-	**0.2**	-	-
1145	**camphene hydrate**	**0.1**	t	-	-	-	-
1158	trans-pinocamphone	t	t	-	-	-	-
1165	borneol	t	t	-	-	t	t
1166	p-mentha-1,5-dien-8-ol	-	-	0.2	0.3	-	-
1066	**coahuilensol**	**t**	**0.1**	**-**	**-**	**-**	**-**
1174	**terpinen-4-ol**	**0.2**	**0.2**	**2.2**	**1.7**	**3.7**	**1.7**
1183	cryptone	-	-	-	**0.3**	-	-
1189	p-cymen-8-ol	t	t	t	t	t	t
1186	α-terpineol	0.1	0.1	0.3	0.3	0.2	t
1195	methyl chavicol	t	-	-	0.3	-	t

KI	compound	mono 15781	mono 15807	mono x scop 15787	mono x scop 15804	scop 15783	scop 15799
1195	cis-piperitol	-	-	t	t	t	t
1198	methyl salicylate	t	0.1	-	-	-	-
1199	safranal	-	-	0.1	-	-	-
1204	verbenone	-	-	-	**0.3**	-	-
1207	trans-piperitol	-	-	t	t	t	t
1219	**coahuilensol, methyl ether**	t	**t**	-	-	-	-
1223	citronellol	-	-	t	0.3	-	0.2
1232	thymol, methyl ether	-	-	1.1	t	-	-
1235	**trans-chrysanthenyl acetate**	**0.2**	**0.1**	-	-	-	-
1239	carvone	t	t	-	-	-	-
1249	piperitone	0.2	t	-	-	t	-
1254	linalyl acetate	-	-	0.1	t	-	-
1274	**pregeijerene B**	**2.0**	**2.7**	**0.6**	**1.2**	**6.3**	**8.7**
1285	safrole	-	-	-	-	-	**15.5**
1287	bornyl acetate	0.6	0.6	0.3	t	0.2	t
1289	thymol	-	-	0.2	t	-	-
1315	(2E,4E)-decadienal	t	t	t	t	t	t
1345	α-terpinyl acetate	-	-	**0.3**	**t**	-	-
1345	α-cubebene	-	-	-	-	-	t
1374	α-copaene	-	-	-	-	t	-
1396	duvalene acetate	t	0.1	t	t	-	-
1391	(2E,4Z)-methyl decadienoate	-	-	t	-	t	-
1403	methyl eugenol	-	-	-	-	t	**2.5**
1407	longifolene	-	-	-	**t**	-	-
1417	(E)-caryophyllene	t	t	0.3	-	0.3	0.1
1451	(Z)-methyl isoeugenol	-	-	-	-	-	0.3
1451	trans-muurola-3,5-diene	-	-	-	-	0.3	-
1452	α-humulene	0.2	t	0.4	-	t	t
1465	cis-muurola-3,5-diene	-	-	-	-	t	t
1468	**pinchotene acetate**	t	**t**	-	-	-	-
1475	trans-cadina-1(6),4-diene	-	-	-	-	t	t
1480	germacrene D	-	-	0.2	-	0.7	0.3
1493	trans-muurola-4(14), 5-diene	-	-	-	-	**0.2**	t
1493	epi-cubebol	-	-	-	-	-	0.1
1500	α-muurolene	0.2	0.2	t	t	0.5	0.3
1513	γ-cadinene	-	-	t	t	1.1	0.5
1521	trans-calamenene	-	-	-	**0.2**		
1522	δ-cadinene	-	-	t	0.3	1.7	1.1
1537	α-cadinene	-	-	-	-	0.1	t
1539	α-copaen-11-ol	t	t	-	0.1	-	0.1
1549	elemol	3.1	0.7	4.7	2.4	5.0	3.9
1555	elemicin	-	-	-	-	-	1.2
1559	**germacrene B**	**0.5**	**0.1**	-	-	-	-
1574	**germacrene D-4-ol**	-	-	**0.5**	**1.4**	**1.6**	**1.7**
1582	caryophyllene oxide	-	-	0.3	-	-	-
1594	ethyl decanoate	-	-	0.2	-	-	-
1607	β-oplopenone	-	-	-	0.2	0.3	0.2
1608	humulene epoxide II	-	-	0.4	-	-	-
1630	γ-eudesmol	2.2	0.4	0.4	0.3	0.4	0.2
1638	epi-α-cadinol	-	-	t	0.2	0.4	0.3
1638	epi-α-muurolol	-	-	t	0.3	0.5	0.2

KI	compound	mono 15781	mono 15807	mono x scop 15787	mono x scop 15804	scop 15783	scop 15799
1644	α-muurolol	-	-	t	t	t	t
1649	β-eudesmol	8.4	0.9	0.9	0.4	0.8	0.3
1652	α-eudesmol	1.4	0.6	0.6	0.5	0.8	0.4
1653	**α-cadinol**	**-**	**-**	**0.5**	**0.4**	**0.6**	**0.5**
1792	**8-α-acetoxyelemol**	**0.8**	**1.0**	**2.2**	**3.9**	**4.9**	**4.5**
1887	oplopanonyl acetate	-	-	-	**0.1**	-	-
1933	cyclohexadecanolide	-	-	-	**0.2**	-	-
1959	hexadecanoic acid	0.6	0.5	0.2	0.1	-	-
2009	manool oxide	t	t	-	-	-	-
2055	abietatriene	t	t	t	0.1	t	t
2087	abietadiene	t	t	0.4	0.2	t	t
2298	4-epi-abietal	-	-	0.4	0.2	0.2	0.1
2312	abieta-7,13-dien-3-one	-	-	-	0.3	t	t
2313	abietal	-	-	-	0.3	-	-
2314	trans-totarol	0.2	t	1.3	-	-	-
2331	trans-ferruginol	0.1	t	0.2	0.1	-	-
2343	4-epi-abietol	t	-	0.2	t	t	t
2401	abietol	-	-	t	t	t	t
2443	methyl neo-abietate	t	t	-	-	-	-

KI = Kovat's Index (linear by temperature programming) om J & W DB-5 column. Values less than 0.05% are denoted as traces (t). Unidentified components less than 0.5% are not reported.